



UNIVERSITI PUTRA MALAYSIA

**EFFECTS OF LACTOBACILLUS STRAINS AS A PROBIOTIC AND A
HYPOLIPIDAEMIC AGENT FOR CHICKENS**

KALAVATHY RAMASAMY

IB 2003 2

**EFFECTS OF *LACTOBACILLUS* STRAINS AS A PROBIOTIC AND A
HYPOLIPIDAEMIC AGENT FOR CHICKENS**

By

KALAVATHY RAMASAMY

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfillment of the Requirement for the Degree of Doctor of Philosophy**

August 2003



Abstract of the thesis submitted to the senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Doctor of Philosophy

EFFECTS OF *LACTOBACILLUS* STRAINS AS A PROBIOTIC AND A HYPOLIPIDAEMIC AGENT FOR CHICKENS

By

KALAVATHY RAMASAMY

August 2003

Chairman : Professor Dr. Ho Yin Wan

Institute : Bioscience

In recent years, there has been considerable interest in the beneficial effects of probiotics (direct-fed microbials, which include *Lactobacillus*) to modulate the lipid metabolism. However, the mechanism(s) involved remains unclear. A series of experiments was carried out to investigate the ability of 12 *Lactobacillus* strains to deconjugate bile salts and to remove cholesterol *in vitro*, and to assess their potential as a probiotic and as a hypolipidaemic agent for broilers and laying hens. Bile salt hydrolase (BSH) activity (resulting in bile salt deconjugation) of intestinal bacteria is closely linked to the lowering of cholesterol. The results of the *in vitro* studies showed that all the 12 *Lactobacillus* strains could deconjugate sodium glycocholate (GCA) and sodium taurocholate (TCA) bile salts, and all the strains, except *L. fermentum* I 24, had a higher affinity for GCA. However, only eight strains could deconjugate sodium taurodeoxycholate (TDCA). This indicates that the BSH of the *Lactobacillus* strains is substrate specific. The 12 *Lactobacillus* strains showed significant differences in their ability to reduce cholesterol from the growth medium (27 to 85 %) with or without bile salt, indicating that bile salt is not a prerequisite for the removal of cholesterol. *Lactobacillus acidophilus* I 16, *L. crispatus* I 12, *L.*

brevis C 17 and I 211, and *L. fermentum* I 24 and I 25 removed cholesterol from the growth medium mainly through assimilation of cholesterol into the cells. On the other hand, *L. brevis* C 1, C 10, I 23 and I 218, and *L. fermentum* C 16 removed cholesterol through both assimilation and co-precipitation of deconjugated bile salt with cholesterol at low pH. The *Lactobacillus* strains assimilated more esterified than non-esterified cholesterol and the assimilated cholesterol was tightly bound to the cells. Cells grown in the presence of cholesterol were more resistant to lysis by sonication than when grown in its absence, suggesting a possible alteration of the cell wall or membrane by the assimilated cholesterol. Cholesterol removal by the *Lactobacillus* strains was also affected by Tween 80.

The feeding trials showed that the supplementation of a mixture of the 12 *Lactobacillus* cultures (LC), as a probiotic for broilers, significantly improved growth equivalent to that provided by the antibiotic, oxytetracycline, but the feed conversion ratio was better in LC-fed broilers. The supplementation of LC also significantly lowered the total cholesterol, low density lipoprotein cholesterol and triglycerides of the serum; the cholesterol of the carcass and liver; abdominal fat deposition; and fat contents of the liver, muscle and carcass of broilers; but there was little effect on the fatty acid compositions of the liver, muscle and carcass.

In laying hens, the supplementation of LC improved the feed efficiency and hen-day egg production during the early stage of the laying cycle, and increased egg weight and influenced a shift from small and medium to large and extra large eggs throughout the laying cycle. However, LC had very little effect on improving the fatty acid composition, and the cholesterol and total fat contents of eggs.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

**KESAN PELBAGAI STRAIN *LACTOBACILLUS* SEBAGAI PROBIOTIK
DAN AGEN HIPOLIPIDIMIK UNTUK AYAM**

Oleh

KALAVATHY RAMASAMY

Ogos 2003

Pengerusi : Profesor Dr. Ho Yin Wan
Institut : Biosains

Sejak kebelakangan ini, kecenderungan untuk menggunakan probiotik (mikrob makanan, termasuk *Lactobacillus*) dalam mengawal atur metabolisme lipid semakin berkembang. Namun demikian, mekanisme yang terlibat masih tidak jelas. Satu siri eksperimen telah dijalankan untuk mengkaji keupayaan 12 strain *Lactobacillus* untuk melakukan dikonjugasi garam hempedu (garam konjugat) dan mengurangkan kolesterol secara *in vitro*, serta kesannya sebagai probiotik dan agen hypolipidimik terhadap ayam pedaging dan ayam penelur. Aktiviti enzim “bile salt hydrolase (BSH)” (yang menyebabkan dikonjugasi garam hempedu) usus berkait rapat dengan pengurangan kolesterol. Hasil kajian *in vitro* menunjukkan bahawa kesemua 12 strain *Lactobacillus* berupaya melakukan dikonjugasi garam “glychocholate” (GCA) dan garam “taurocholate” (TCA), dan kesemua strain, kecuali *L. fermentum* I 24, menunjukkan afiniti yang lebih tinggi terhadap GCA. Tetapi hanya lapan strain berupaya melakukan dikonjugasi garam “taurodeoxycholate”. Ini menunjukkan bahawa aktiviti BSH *Lactobacillus* adalah spesifik substrat. Duabelas strain *Lactobacillus* ini juga menunjukkan keupayaan untuk mengurangkan kolesterol dari media kultur (25 hingga 85 %) yang ada atau tiada garam hempedu. Pengurangan kolesterol dari media kultur oleh *L. acidophilus*

I 16, *L. crispatus* I 12, *L. brevis* C 17 dan I 211, dan *L. fermentum* I 24 dan I 25 adalah terutamanya melalui asimilasi kolesterol oleh sel. Pengurangan kolesterol oleh *L. brevis* C 1, C 10, I 23 dan I 218, dan *L. fermentum* C 16 pula, adalah melalui asimilasi dan juga ko-mendakan garam hempedu tak berkonjugat bersama kolesterol pada pH yang rendah. Strain *Lactobacillus* mengasimilasi lebih banyak kolesterol ester berbanding dengan kolesterol bebas dan kolesterol yang diasimilasi didapati terikat dengan kuat pada sel. Sel yang ditumbuhkan bersama kolesterol juga lebih resistan kepada sonikasi, mencadangkan bahawa pengubahsuaian pada dinding atau membran sel berlaku setelah mengasimilasi kolesterol. Pengurangan kolesterol oleh strain *Lactobacillus* juga bergantung pada Tween 80.

Hasil kajian *in vivo* menunjukkan bahawa campuran 12 strain *Lactobacillus* (LC), sebagai probiotik pada ayam pedaging dapat meningkatkan berat badan sama seperti antibiotik “oxytetracycline”, tetapi kadar penukaran makanan ayam adalah lebih baik pada ayam yang di beri LC. Penambahan LC pada ayam juga dapat menurunkan paras “total” kolesterol, “low density lipoprotein” kolesterol dan trigliserida di serum; kandungan kolesterol pada karkas dan hati; lemak berlebihan pada bahagian abdomen; dan kandungan lemak pada hati, otot dan karkas; tetapi tidak berupaya mengubah profil asid lemak pada hati, otot dan karkas.

Ayam penelur yang di beri LC dapat meningkatkan kadar penukaran makanan dan produksi telur pada peringkat awal peneluran serta dapat menghasilkan telur yang lebih berat dan saiz yang lebih besar sepanjang proses peneluran. Namun demikian, LC kurang berkesan untuk mengubah profil asid lemak, atau menurunkan paras kolesterol dan lemak di telur.

ACKNOWLEDGEMENTS

I wish to express my deep appreciation and most sincere gratitude to the chairman of the supervisory committee, Professor Dr. Ho Yin Wan, for her invaluable guidance and advice, endless support, patience, and encouragement throughout the duration of this study and for her critical analysis, constructive criticism and helpful suggestions during the preparation of my thesis.

I am deeply grateful and indebted to Associate Professor Dr. Norhani Abdullah and Dr. Clemente Michael Wong, who are members of the supervisory committee, for their kind assistance, advice and guidance throughout the course of my work and in the preparation of the thesis.

Special appreciation goes to Tan Sri Dato Dr. Syed Jalaludin Syed Salim (who was a member of the supervisory committee till his retirement in 2001) for his wise counsel, support and constant encouragement.

My heartfelt appreciations are extended to Madam Haw Ah Kam, Mr. Khairul Kamar Bakri, Mr. Nagayah Muniandy, Mr. Jivanathan Arumugam and Mr. Paimon Lugiman, staff of the Enzyme and Microbial Technology Laboratory, and Mr. Saparin Denim and Mr. Ibrahim Mohsin, staff of Animal Nutrition Laboratory, for their technical support and kind assistance. Thanks are also due to Dr. Goh Yong Meng for his assistance on the preparation of samples for the fatty acid determination using Gas Chromatography.

I wish to extend my sincere thanks to my post graduate friends Chin Chin, Latiffah, Lan, Darlis, Vicky, Wan, Thongsuk, Lee, Pit Kang and Sidieg for their friendship, support, encouragement and their sense of humor that made the many hours in the laboratory very pleasant, which contributed to the successful completion of this work.

Finally, very special thanks are due to my family for their unconditional love, untiring patience, support and encouragement, which inspired and motivated me throughout the course of this study.

I certify that an Examination Committee met on 6th August 2003 to conduct the final examination of Kalavathy Ramasamy on her Doctor of Philosophy thesis entitled “Effects of *Lactobacillus* Strains as a Probiotic and a Hypolipidaemic Agent for Chickens” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

ABDUL RAZAK ALIMON, Ph.D.

Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

HO YIN WAN, Ph.D.

Professor
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Member)

NORHANI ABDULLAH, Ph.D.

Associate Professor
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Member)

CLEMENTE MICHAEL WONG, Ph.D.

Faculty of Food Science and Biotechnology
Universiti Putra Malaysia
(Member)

HYUNG TAI SHIN, Ph.D.

Professor
Department of Food and Bioresources
Faculty of Life Science and Technology
Sung Kyun Kwan University
300 Chunchun-Dong, Jangan-Ku
Suwon 440-746, Republic of Korea
(Independent Examiner)



GULAM RUSUL RAHMAT ALI, Ph.D.

Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 4 SEP 2003

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

HO YIN WAN, Ph.D.

Professor
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Chairman)

NORHANI ABDULLAH, Ph.D.

Associate Professor
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Member)

CLEMENTE MICHAEL WONG, Ph.D.

Faculty of Food Science and Biotechnology
Universiti Putra Malaysia
(Member)



AINI IDERIS, Ph.D.

Professor/Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: **16** SEP 2003

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



KALAVATHY RAMASAMY

Date: 22/9/03

TABLE OF CONTENTS

	Page
ABSTRACT	ii
ABSTRAK	iv
ACKNOWLEDGEMENTS	vi
APPROVAL	viii
DECLARATION	x
LIST OF TABLES	xiv
LIST OF FIGURES	xvii
LIST OF ABBREVIATIONS	xx

CHAPTER

1	INTRODUCTION	1
2	LITERATURE REVIEW	5
	2.1 Poultry Industry	5
	2.2 Global Challenges in the Modern Poultry Industry	6
	2.3 Lipids and Human Health	8
	2.4 Lipids in Broiler Meat and Eggs	10
	2.4.1 Lipid	10
	2.4.2 Fat deposition, Cholesterol and Fatty Acids	12
	2.4.3 Strategies in Improving the Lipid Content in Broiler Meat	14
	2.4.4 Strategies in Improving the Lipid Content in Eggs	16
	2.4.5 Biological Methods in Improving Lipids in Broiler Meat and Eggs	17
	2.5 Antibiotics in Poultry Production : Benefits and Risks	18
	2.6 Probiotics	22
	2.6.1 Contributions of the Intestinal Microflora	22
	2.6.2 Definition	23
	2.6.3 Probiotics Currently in Use	24
	2.6.4 Mode of Action of Probiotics	25
	2.6.5 Selection Criteria for Probiotics	28
	2.6.6 Benefits of Probiotics on Poultry Performance	28
	2.7 Hypocholesterolaemic Effect of Lactic Acid Bacteria	33
	2.8 Bile Salt Deconjugation of Lactic Acid Bacteria	36
	2.8.1 Enterohepatic Circulation of Bile Acids	36
	2.8.2 Significance of Bile Salt Deconjugation by the Lactic Acid Bacteria	37
3	BILE SALT HYDROLASE ACTIVITY OF <i>LACTOBACILLUS</i> CULTURES FROM CHICKEN	40
	3.1 Introduction	40
	3.2 Materials and Methods	41

3.2.1	Source and Maintenance of <i>Lactobacillus</i> Strains	41
3.2.2	Bile Salt Deconjugation by <i>Lactobacillus</i> Strains	42
3.2.3	Kinetics of Bile Salt Deconjugation	45
3.2.4	Bile Tolerance Test	46
3.3	Results	46
3.3.1	Morphological Characteristics	46
3.3.2	Bile Salt Deconjugation by <i>Lactobacillus</i> Strains	47
3.3.3	Kinetic Parameters of Bile Salt Deconjugation	61
3.3.4	Bile Tolerance	71
3.4	Discussion	72
4	CHOLESTEROL-REDUCING ABILITY OF <i>LACTOBACILLUS</i> STRAINS <i>IN VITRO</i> AND THE MECHANISM (S) INVOLVED	79
4.1	Introduction	79
4.2	Materials and Methods	80
4.2.1	Preliminary Study of Cholesterol Reduction by 12 <i>Lactobacillus</i> Strains	80
4.2.2	Effects of Various Bile Salt Concentrations on the Reduction of Cholesterol	81
4.2.3	Effects of Various Concentrations of Tween 80 on the Reduction of Cholesterol	82
4.2.4	Effect of Cholesterol on Growth of <i>Lactobacillus</i> Strains	83
4.2.5	Quantitative Analysis of Cholesterol in the Culture Supernatant and Bacterial Cell Pellet of Three <i>Lactobacillus</i> Strains	84
4.2.6	Qualitative Analysis on the Assimilation of Cholesterol by <i>Lactobacillus</i> Strains	85
4.2.7	Effects of Cholesterol and Bile Salts on Lysis of <i>Lactobacillus</i> by Sonication	86
4.2.8	Effects of pH and Bile Salts on Solubility of Cholesterol	87
4.3	Results.....	88
4.3.1	Reduction of Cholesterol in Growth Media by 12 <i>Lactobacillus</i> Strains	88
4.3.2	Effects of Bile Salt on Cholesterol Reduction	90
4.3.3	Effects of Concentrations of Tween 80 on Cholesterol Reduction	91
4.3.4	Effect of Cholesterol on Growth of <i>Lactobacillus</i> Strains	93
4.3.5	Quantitative Analysis of Cholesterol in the Culture Supernatant and Cell Pellet of <i>Lactobacillus</i> Strains ...	96
4.3.6	Qualitative Analysis of Cholesterol in Cell Pellets of <i>Lactobacillus</i> Strains	96
4.3.7	Effect of Cholesterol and Bile Salts on Lysis of <i>Lactobacillus</i> by Sonication	104
4.3.8	Influence of pH and Bile Salts on Solubility of Cholesterol	105
4.4	Discussion	107

5	EFFECTS OF <i>LACTOBACILLUS</i> CULTURES ON BROILER CHICKENS	117
5.1	Introduction	117
5.2	Materials and Methods	118
5.2.1	Animals and Rearing Management	119
5.2.3	Experiment II	122
5.2.4	Statistical Analysis	129
5.3	Results	129
5.3.1	Experiment I	129
5.3.2	Experiment II	132
5.4	Discussion	142
6	EFFECTS OF <i>LACTOBACILLUS</i> CULTURES ON LAYING HENS	157
6.1	Introduction	157
6.2	Materials and Methods	158
6.2.1	Animals and Rearing Management	158
6.2.2	Dietary Treatment	159
6.2.3	Layer Performance and Production	159
6.2.4	Egg Quality and Egg Storage Test	161
6.2.5	Yolk Total Lipids, Fatty Acid Composition and Cholesterol	163
6.2.6	Statistical Analysis	164
6.3	Results	164
6.3.1	Ambient Temperature and Relative Humidity	164
6.3.2	Layer Performance and Production	165
6.3.3	Egg Quality and Storage Test	175
6.3.4	Egg Yolk Cholesterol, Total Lipids and Fatty Acid Composition	180
6.4	Discussion	185
7	GENERAL DISCUSSION AND CONCLUSIONS	194
7.1	General Discussion	194
7.2	Conclusions	206
	BIBLIOGRAPHY	208
	VITA	234

LIST OF TABLES

Table		Page
1	Possible modes of actions of probiotics	27
2	Criteria for an effective probiotic strain	29
3	Hypocholesterolaemic effects of lactic acid bacteria on various hosts	34
4	<i>Lactobacillus</i> strains (from chicken) used in the study	42
5	Bile salt hydrolase (BSH) activity of <i>Lactobacillus</i> strains on MRS + sodium taurodeoxycholate (MRS + TDCA) agar plates	51
6	Comparison of the deconjugation of sodium taurocholate and sodium glycocholate by 12 <i>Lactobacillus</i> strains	59
7	Kinetics of bile salt deconjugation by <i>L. brevis</i> C 10 from 2 to 24 h of incubation	63
8	Kinetics of bile salt deconjugation by <i>L. fermentum</i> C16 from 2 to 24 h of incubation	65
9	Kinetics of bile salt deconjugation by <i>L. acidophilus</i> I 26 from 2 to 24 h of incubation	67
10	Kinetics of bile salt deconjugation by <i>L. acidophilus</i> I 16 from 2 to 24 h of incubation	69
11	Growth of <i>Lactobacillus</i> strains in MRS broth and MRS with 0.3 % bile salt at 4 h	71
12	Reduction of cholesterol in growth media by 12 <i>Lactobacillus</i> strains	89
13	Effects of bile salt concentrations on cholesterol reduction by <i>Lactobacillus</i> strains	91
14	Effects of Tween 80 concentrations on cholesterol reduction by <i>Lactobacillus</i> strains	92
15	Comparison of growth of three <i>Lactobacillus</i> strains in various growth media	94
16	Percentages of cholesterol reduced in the MRSC and MRSBC supernatants and percentages of cholesterol assimilated in the cell pellets of three <i>Lactobacillus</i> strains	97

17	Fluorescence intensity of cell pellets of <i>Lactobacillus</i> strains grown in various media and stained with filipin	98
18	Fluorescence intensity of cell pellets of <i>Lactobacillus</i> strains grown in various media and stained with Nile Red	101
19	Effects of cholesterol and bile salts on lysis of <i>Lactobacillus</i> by sonication	105
20	Composition of the basal diets	121
21	Effects of <i>Lactobacillus</i> cultures (LC) or oxytetracycline on body weight, weight gain, feed intake and feed to gain ratio of broiler chickens for 42 days	131
22	Effects of <i>Lactobacillus</i> cultures (LC) on body weight, weight gain and feed to gain ratio of broiler chickens for 42 days	133
23	Percentage by weight of organs from broiler chickens fed diets with or without <i>Lactobacillus</i> cultures (LC) from 21 to 42 days of age ...	134
24	Abdominal fat deposition of broiler chickens fed with or without <i>Lactobacillus</i> cultures (LC) from 21 to 42 days of age	135
25	Serum lipid concentrations in broiler chickens fed with or without <i>Lactobacillus</i> cultures (LC) from 21 to 42 days of age	140
26	Effects of <i>Lactobacillus</i> cultures (LC) on cholesterol contents of carcass, liver and muscle of broiler chickens at 42 days of age	141
27	Effects of <i>Lactobacillus</i> cultures (LC) on fat contents of carcass, liver and muscle of broiler chickens at 42 days of age	141
28	Fatty acid composition of carcass from broilers supplemented with or without <i>Lactobacillus</i> cultures (LC) at 42 days of age	143
29	Fatty acid composition of liver from broilers supplemented with or without <i>Lactobacillus</i> cultures (LC) at 42 days of age	144
30	Fatty acid composition of muscle from broilers supplemented with or without <i>Lactobacillus</i> cultures (LC) at 42 days of age	145
31	Composition of the basal diet	160
32	Egg size distribution	161
33	Effects of <i>Lactobacillus</i> cultures (LC) on feed intake, feed efficiency, hen-day egg production and mortality of laying hens from 20 to 68 weeks of age	166

34	Effects of <i>Lactobacillus</i> cultures (LC) on egg weight and egg mass of laying hens from 20 to 68 weeks of age	170
35	Effects of <i>Lactobacillus</i> cultures (LC) on egg size of laying hens from 20 to 68 weeks of age	173
36	Effects of <i>Lactobacillus</i> cultures (LC) on egg quality of hens from 20 to 35 weeks of age	176
37	Cholesterol contents of eggs from hens supplemented with or without <i>Lactobacillus</i> cultures (LC) at 24, 28, 32 and 68 weeks of age	181
38	Total lipid contents of eggs from hens supplemented with or without <i>Lactobacillus</i> cultures (LC) at 24, 28 and 32 weeks of age	181
39	Fatty acid composition of eggs from hens supplemented with or without <i>Lactobacillus</i> cultures (LC) at 24 weeks of age	182
40	Fatty acid composition of eggs from hens supplemented with or without <i>Lactobacillus</i> cultures (LC) at 28 weeks of age	183
41	Fatty acid composition of eggs from hens supplemented with or without <i>Lactobacillus</i> cultures (LC) at 32 weeks of age	184

LIST OF FIGURES

Figure		Page
1	Cell morphology of <i>Lactobacillus</i> strains observed using light microscopy	48
2	Colonies of <i>Lactobacillus</i> strains on MRS agar	49
3	Plate assay showing high bile salt hydrolase (BSH) activity of <i>L. fermentum</i> C 16	52
4	Plate assay showing high bile salt hydrolase (BSH) activity of <i>L. brevis</i> C 1	53
5	Plate assay showing high bile salt hydrolase (BSH) activity of <i>L. brevis</i> C 10	54
6	Plate assay showing no bile salt hydrolase (BSH) activity of <i>L. brevis</i> C 17	55
7	Plate assay showing low bile salt hydrolase (BSH) activity of <i>L. crispatus</i> I 12 and <i>L. brevis</i> I 23	56
8	Plate assay showing absence of bile salt hydrolase (BSH) activity in <i>L. fermentum</i> I 24 and <i>L. acidophilus</i> I 26	57
9	Precipitates due to bile salt hydrolase (BSH) activity as observed under the light microscope	58
10	Deconjugation of sodium glycocholate (GCA) and sodium taurocholate (TCA) by <i>Lactobacillus</i> strains	60
11	Growth and changes in pH, and disappearance of conjugated bile salt in MRS broth supplemented with sodium taurocholate (TCA) and sodium glycocholate (GCA) of <i>L. brevis</i> C 10 from 0 to 24 h of incubation	64
12	Growth and changes in pH, and disappearance of conjugated bile salt in MRS broth supplemented with sodium taurocholate (TCA) and sodium glycocholate (GCA) of <i>L. fermentum</i> C 16 from 0 to 24 h of incubation	66
13	Growth and changes in pH, and disappearance of conjugated bile salt in MRS broth supplemented with sodium taurocholate (TCA) and sodium glycocholate (GCA) of <i>L. acidophilus</i> I 26 from 0 to 24 h of incubation	68

14	Growth and changes in pH, and disappearance of conjugated bile salt in MRS broth supplemented with sodium taurocholate (TCA) and sodium glycocholate (GCA) of <i>L. acidophilus</i> I 16 from 0 to 24 h of incubation	70
15	Growth of <i>L. brevis</i> C 10, <i>L. acidophilus</i> I 26 and <i>L. acidophilus</i> I 16 in four different media with or without cholesterol	95
16	Fluorescence micrographs of cell pellets of <i>L. acidophilus</i> I 26 stained with filipin	99
17	Fluorescence micrographs of cell pellets of <i>L. acidophilus</i> I 26 stained with Nile Red	102
18	Fluorescence micrographs of cell pellets of <i>L. brevis</i> C 10 stained with Nile Red	103
19	Influence of pH and bile salts on solubility of cholesterol	106
20	Abdominal fat depositions of broiler chickens at 42 days of age fed without or with <i>Lactobacillus</i> cultures	136
21	Fat depositions at different areas in broiler chickens at 42 days of age fed without or with <i>Lactobacillus</i> cultures	137
22	Fat deposited on the skin of broiler chickens at 42 days of age fed without or with <i>Lactobacillus</i> cultures	138
23	Effect of <i>Lactobacillus</i> cultures (LC) on feed efficiency of laying hens from 20 to 68 weeks of age	167
24	Effect of <i>Lactobacillus</i> cultures (LC) on egg production of laying hens from 20 to 68 weeks of age	168
25	Effect of <i>Lactobacillus</i> cultures (LC) on egg weight of laying hens from 20 to 68 weeks of age	171
26	Effect of <i>Lactobacillus</i> cultures (LC) on egg mass of laying hens from 20 to 68 weeks of age	172
27	Effect of <i>Lactobacillus</i> cultures (LC) on egg size of laying hens from 20 to 44 weeks of age	174
28	Effect of <i>Lactobacillus</i> cultures (LC) on egg size of laying hens from 45 to 68 weeks of age	174
29	Effect of <i>Lactobacillus</i> cultures (LC) and storage time on internal egg quality of hens from 20 to 68 weeks of age	177

30	Internal egg quality of a fresh egg and an egg that was stored for 7 days from a top view	178
31	Internal egg quality of a fresh egg (A) and an egg that was stored for 7 days (B) from a lateral view	179

LIST OF ABBREVIATIONS

AAP	Aminoantipyrine
ADP	Adenosine diphosphate
AFTA	Asean Free Trade Centre
AOAC	Association of Official Analytical Chemists
ATP	Adenosine triphosphate
BSH	Bile salt hydrolase
CFU	Colony forming unit
cm	centimetre
CP	Cell pellet
d	Day
FAME	Fatty acid methyl ester
FAO	Food and Agriculture Organisation
FDA	Food and Drug Administrations
g	gram
GC	Gas Chromatography
GCA	Sodium glychocholate
GRAS	Generally Recognized as Safe
h	hour
H ₂ O ₂	Hydrogen peroxide
HACCP	Hazzard Analysis Critical Control Points
HBA	Hydroxybenzoic acid
HDL	High density lipoprotein
HMG CoA	Hydroxymethylglutaryl coenzyme A
HPLC	High Performance Liquid Chromatography
HU	Haugh unit
IDL	Intermediate density lipoprotein
IU	International Unit
kg	kilogram
KIC	α -ketoisocaproic acid
KOH	Potassium hydroxide
l	litre
LABIP	International Platform for Lactic Acid Bacteria
LC	A mixture of 12 <i>Lactobacillus</i> cultures
LDL	Low density lipoprotein
M	Molar
m	metre
mg	milligram
min	minute
MJ	megajoules
mRNA	Messenger Ribonucleic Acid
MRS	Man Rogoso Sharpe
MRSB	MRS containing bile salt
MRSC	MRS containing cholesterol
MRSBC	MRS containing bile salt and cholesterol
MRS-TDCA	MRS agar supplemented with 0.5 % sodium taurodeoxycholate
MUFA	Monounsaturated fatty acids
NaCl	Sodium chloride
NaOH	Sodium hydroxide

ND	No data
OD	Optical density
OTC	Oxytetracycline
PTA	Phototungstic Acid
PPLO	Pleuropneumonia-like organism
PUFA	Polyunsaturated fatty acids
SAS	Statistical Analysis Software
SCFA	Short chain fatty acids
SFA	Saturated fatty acids
ST	Supernatant
TCA	Sodium taurocholate
TDCA	Sodium taurodeoxycholate
tRNA	Transfer Ribonucleic Acid
UFA	Unsaturated fatty acids
µg	microgram
µl	microlitre
VLDL	Very low density lipoprotein
W	Watt
WHO	World Health Organisation

CHAPTER 1

INTRODUCTION

The worldwide poultry industry provides a substantial proportion of the nutritional requirement of the human population. Poultry meat is perceived to be lean and low in cholesterol, so it may come as a surprise to learn that poultry scientists and producers are increasingly concerned about the amount of fat present in chicken meat. Chambers *et al.* (1981), Lin (1981) and Havenstein *et al.* (1994) reported that, as a result of selection strategy for body weight gain or growth rate, modern fast-growing broilers have been found to contain about four times higher amounts of abdominal fat than those in the 1960s. Eggs have also been viewed with suspicion today because of their high cholesterol content (Stadelman, 1999). In the US, egg consumption has declined from 256 eggs per capita per year in 1985 to 235 in 1995 (USDA, 1997). The lipid composition of animal products is a primary consumer concern as high fat and cholesterol intakes have been implicated to contribute to coronary heart disease, the most common chronic illness in developed countries. To the poultry producers, on the other hand, excess fat is an economic burden, as fat is lost during processing of the carcass or of the meat, resulting in lower meat yields and, furthermore, the discarded abdominal fat and visceral fat increases waste management problems. This has put the poultry production system under pressure and, therefore, much attention is now directed towards producing healthier meat and eggs such that the lipid fraction is improved (reduced cholesterol and fat and improvement of the fatty acid make-up). Animal feed strategies, genetic selections, and gene manipulation are some of the techniques that have been developed to alter the lipid composition in broilers (Jiménez-Colmenero, 2000) and

egg yolk (Hargis, 1988). However, very often these techniques are cost prohibitive or may impair performances and, therefore, not economically feasible to be applied at commercial scale. Animal welfare and environmental issues may also be linked in the application of these techniques.

Performance and economic returns are one of the main concerns of the commercial poultry industry. To achieve these goals, very often, intensive farming systems are adopted, subjecting broilers and laying hens to various stressful situations. Stress may lower the body's defense mechanism and create an imbalance in the intestinal microflora (Fuller, 1999), which in turn increases susceptibility to infectious diseases, resulting in poor performance. Efforts to prevent or reduce avian diseases include improved management practices, but inevitably at a cost, because this requires high quality feed manufacturing and feeding systems where the environment and the feed are relatively pathogen-free (Zhang-Barber *et al.*, 1999). The benefits of incorporating antibiotic growth promoters in animal feeds are well substantiated (Bedford, 2000). These products have been used for many years by the poultry industry and have proved to be an effective way of enhancing animal status, uniformity and production efficiency. The Union of Concerned Scientists recently estimated that, each year, 11.2 million kg of antimicrobials are given to animals for non-therapeutic purposes, and 900,000 kg are given for therapy, thus, it is fair to state that substantial amounts of antimicrobials are administered to food animals for growth promotion and feed efficiency in the absence of known disease (Gorbach, 2001). However, the use of antibiotics as growth promoters is severely restricted or totally banned in poultry production in many countries, largely because of concern on the development of resistant bacterial strains and residual toxicity in